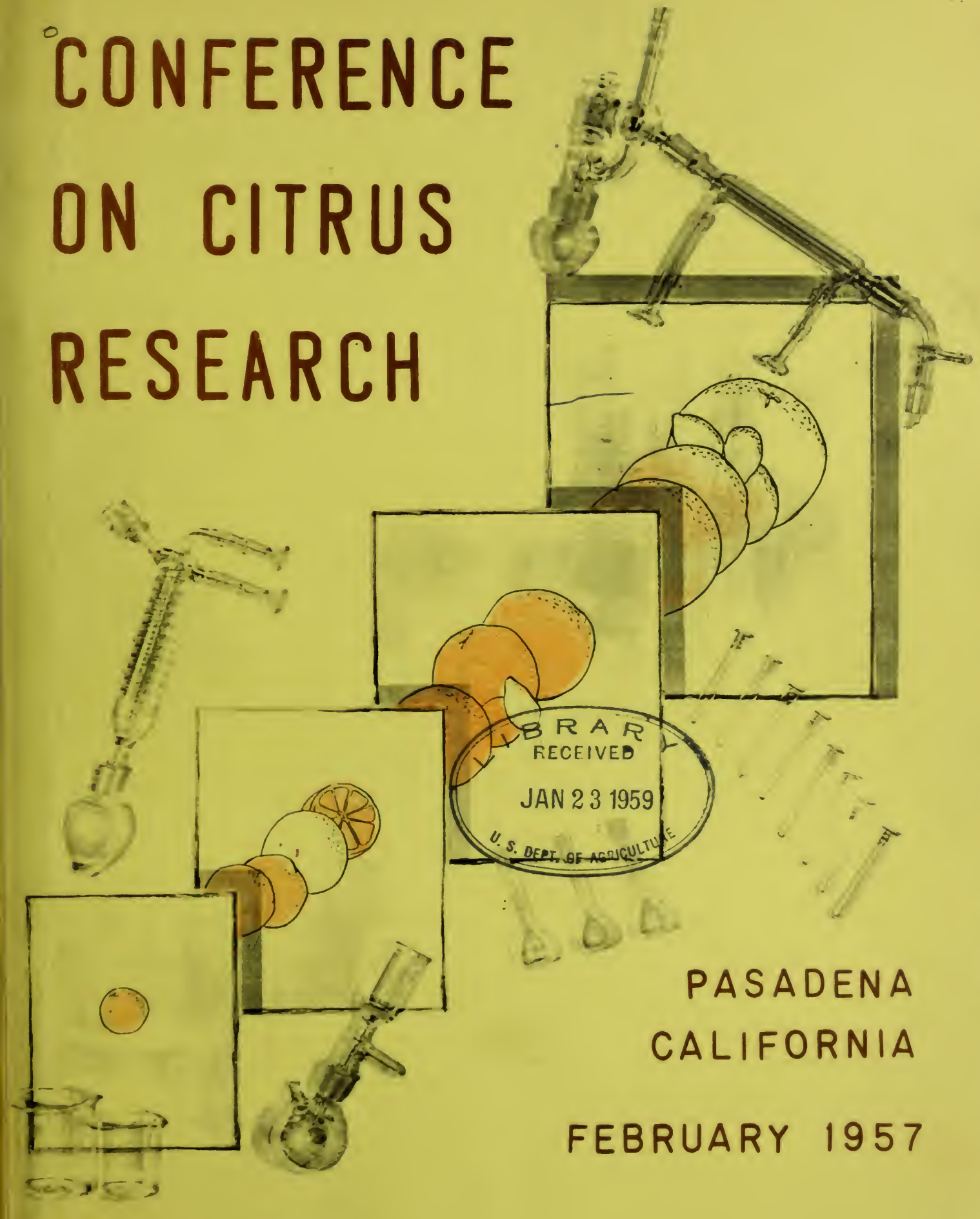
CONFERENCE ON CITRUS RESEARCH



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UNITED STATES DEPARTMENT OF AGRICULTURE
U.S. Agricultural Research Service,
Western Utilization Research Branch, mtd.c

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PROGRAM AND ABSTRACTS OF PAPERS.

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CITRUS RESEARCH CONFERENCE.

February 5, 1957

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
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Pasadena, 5, Calif~~ornia~~

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in California and Arizona the latest results of research on the chemistry and technology of citrus fruits and fruit products carried on by laboratories of the Agricultural Research Service, U.S. Department of Agriculture, and by State laboratories. The following research agencies are participating in this year's Conference:

U. S. Department of Agriculture, Agricultural Research Service

Western Utilization Research Branch
Western Regional Research Laboratory (Branch Headquarters), Albany, California
Fruit and Vegetable Chemistry Laboratory, Pasadena, California

Southern Utilization Research Branch
U. S. Citrus Products Station, Winter Haven, Florida
U. S. Fruit and Vegetable Products Laboratory, Weslaco, Texas

Horticultural Crops Research Branch
U. S. Date Field Station, Indio, California

University of California Agricultural Experiment Station
Citrus Experiment Station, Riverside, California

Lemon Products Advisory Board, Los Angeles, California
(Under a Memorandum of Understanding this Board conducts research on the chemistry of lemon oils in cooperation with the U. S. Department of Agriculture at the Fruit and Vegetable Chemistry Laboratory, Pasadena, California.)

P R O G R A M --- C I T R U S R E S E A R C H C O N F E R E N C E

Tuesday, February 5, 1957

9:30 a.m.

L U N C H

FURTHER STUDIES ON THE CAROTENOID POLYOLS OF ORANGE JUICE

A. Laurence Curl

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Research Laboratory, Albany, Calif.

Earlier work showed that countercurrent distribution in a glass Craig apparatus was of value in the fractionation of the carotenoids of Valencia orange juice. When the solvent system consisting of petroleum ether and 90% methanol was used, the carotenoids were separated into 3 fractions: hydrocarbons, monols, and diols-polyols. The first two of these were then readily separated by chromatography on magnesia columns into 5 and 2 constituents, respectively. The third fraction was much more complex and was not satisfactorily resolved by chromatography.

When the solvent system consisting of benzene, petroleum ether, and 87% methanol (1 to 1 to 1.15 by volume) was used, the carotenoids were separated into 5 fractions: hydrocarbons-monols, diols, monoether diols, diether diols, and polyols. On chromatography the second, third, and fourth fractions were readily separated into 2, 3, and 6 constituents, respectively. The last fraction yielded 8 components but the chromatography was not as clean-cut and some of the components were probably impure. At least 6 of these were apparently previously undescribed carotenoids, for which the names valenciaxanthin, valenciachrome a, valenciachrome b, sinensiaxanthin, sinensiachrome, and trollein were proposed. The other two components resembled trollixanthin and trollichrome, the structures of which were recently elucidated by Karrer and co-workers.

Earlier attempts to fractionate the carotenoid polyols by means of countercurrent distribution, with solvent systems such as benzene, petroleum ether, and 75% methanol were unsuccessful due to the formation of very persistent emulsions. More recent investigation of many other solvent systems led to the discovery of several in which the carotenoid polyol fraction had a distribution coefficient of around 1 and in which emulsion formation was not a serious problem. The system consisting of petroleum ether, acetone, methanol, and water (1.25 to 1 to 0.1 to 0.65, by volume) was considered the most satisfactory from the standpoints of maximum fractionation of the carotenoid polyols and minimum emulsion formation. In a 100-transfer run with this system using carotenoid polyols from orange juice, 3 major and 2 minor peaks were observed.

Countercurrent distribution runs were then made with this system, using bands obtained on chromatography of the carotenoid polyol fraction. By this means it was discovered that the trollein, trollixanthin-like, and trollichrome-like fractions each consisted of a pair of substances with very similar spectral absorption curves but with considerably different N_{100} values (N_{100} is the tube number of the maximum based on 100 transfers), which were, respectively: 73 and 54; 53 and 30; and 57 and 38; the differences in the N_{100} values in each pair were around 20. This may be due to the first member of each pair being a triol and the second a tetrol. An additional constituent of the polyol fraction was also discovered, so that this fraction is now known to contain at least 12 constituents.

PARTITION SEPARATION OF CAROTENOIDS BY MEANS OF SILICA GEL METHANOL COLUMNS

Albert E. Purcell

Southern Utilization Research Branch, Fruit and Vegetable Products Laboratory, Weslaco, Texas

In attempting to study the carotenoid pigments of Texas red grapefruit, difficulty is experienced, since the two major pigments, beta carotene and lycopene, comprise at least 90% of the carotenoid fraction. The remainder of the pigment fraction is a complex mixture of carotenoids present in very small amounts. In the usual chromatographic procedures some of the minor pigments absorb between the carotene and lycopene, and others absorb very closely above lycopene. Because of the small amounts present, the various minor pigments are often lost on columns that are of sufficient size to separate the major pigments.

The classical solution to this problem has been to wash a solution of the pigment mixture several times with an immiscible solvent which will separate the pigments according to their relative solubilities in each solvent. This preliminary phasic separation greatly simplifies subsequent separation. Curl and Bailey (WURB) have refined this immiscible solvent separation technique by use of a Craig countercurrent separation apparatus. It has been found that columns of silica gel treated with methanol can effectively separate the pigments into several fractions according to their relative solubilities in hexane and methanol. The results are similar to those achieved by Curl with countercurrent separation. To date authentic samples of carotene (hydrocarbons), monohydroxy carotenoids, and polyhydroxy carotenoids have been separated.

RECENT WORK ON IDENTIFICATION OF THE FLAVONOIDS OF LEMONS

R. M. Horowitz and B. Gentili

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Vegetable Chemistry Laboratory, Pasadena, Calif.

Paper chromatograms of extracts of lemon peel show the presence of at least 5 major flavonoid constituents. Two of these are flavanones, while the others are either flavones or flavonols. Other flavonoids occur regularly in these extracts but in smaller concentration. Hesperidin has long been recognized as a constituent of lemons, and the presence of diosmin (3',5,7-trihydroxy-4'-methoxyflavone-7-rhamnoglucoside) has been reported recently by this laboratory.

The presence of eriodictyol (5,7,3',4'-tetrahydroxyflavanone) in lemons has now been confirmed. It occurs in the plant as a glycoside and was obtained in this work as the pure, crystalline aglycone upon hydrolyzing a mixture of lemon glycosides. The identity of this compound was established as follows: It reacted with sodium borohydride to give a purple color, and it reduced Tollens' reagent. These tests demonstrated that the compound was a flavanone and contained an orthodihydroxy group, respectively. The compound yielded a tetra-acetate and an oxime, both of which were analyzed. Upon treatment of the tetra-acetate with N-bromsuccinimide, luteolin was formed which was identified by comparison with an authentic sample. Finally, a sample of eriodictyol was synthesized by demethylating hesperitin. Direct comparison of this material and its derivatives with the material isolated from natural sources showed them to be identical.

A small quantity of a new flavonol, occurring with eriodictyol, has recently been isolated from the hydrolyzed lemon glycosides. This flavonol has not been reported in the literature. An investigation of its structure is under way.

The previously described sodium borohydride test for flavanones has been extended by testing its reaction with several naturally occurring flavanones not available earlier. The application of this test to the question of whether oranges contain eriodictyol will be discussed.

Publication: Flavonoids of Citrus. I. The Isolation of Diosmin from Lemons. R. M. Horowitz, Jour. Organic Chemistry, 21, 1184 (1956).

METABOLISM OF CITRUS FLAVONOIDS

Floyd DeEds

Western Utilization Research Branch, Western Regional Research Laboratory, Albany, Calif.

At the 1956 Citrus Conference a report was given on the metabolic fate of homoeriodictyol and the citrus flavonoids diosmin, its aglycone diosmetin, naringin, its aglycone naringenin, and the aglycone hesperetin. Eriodictyol, known to occur in lemons, was not available yet for study, but its methyl derivative, homoeriodictyol, was investigated. All these flavonoids are flavanones which are degraded in the animal body by splitting of the flavonoid molecule to yield phenylpropionic acid derivatives. It was pointed out that in contrast to the flavanones the flavonol quercetin is degraded to yield phenylacetic acid derivatives.

The metabolic fate of the glycoside hesperidin was not reported because clear evidence of its absorption and metabolism had not been obtained. By placing rats on a diet of casein and sucrose and by administering the hesperidin by stomach tube it has been shown that absorption occurs followed by the urinary excretion of hesperetin, meta-hydroxyphenylpropionic acid, and a conjugate of the aglycone hesperetin. These are the same products as those excreted after oral administration of hesperetin.

A small sample of eriodictyol prepared synthetically proved to be the first flavonoid studied to date that shows a species difference in its metabolic fate. When fed to rabbits eriodictyol caused the urinary excretion of meta-hydroxyphenylpropionic acid, dihydrocaffeic acid, dihydroferulic acid, and meta-hydroxybenzoic acid. The latter compound may be the result of shortening of the 3-carbon side chain by beta-oxidation. Feeding eriodictyol to rats caused the urinary excretion of meta-hydroxyphenylpropionic acid, dihydrocaffeic acid, dihydroferulic acid, and homoeriodictyol, but no meta-hydroxybenzoic acid. To excrete homoeriodictyol the rat must have methylated the 3'-hydroxy group of eriodictyol.

The ability of diosmetin and eriodictyol to function as antioxidants and prolong the characteristic relaxation of surviving excised intestinal smooth muscle by epinephrine has been studied. Both compounds were found to be relatively ineffective. These results are in accord with the reported observations of Wilson and DeEds (Journal of Pharmacology and Experimental Therapeutics, Vol. 95, 399, 1949) and Clark and Geissman (Federation Proceedings, Vol. 7, 21, 1948), who showed that hydroxyl groups in the 3',4' positions and a double bond between the numbers 2 and 3 carbon atoms are important to marked antioxidant activity. Diosmetin possesses the double bond but lacks the two free hydroxy groups. Eriodictyol has the two free hydroxyl groups but the double bond is missing.

. PROGRESS ON BUTTERMILK AND BITTER FLAVORS OF CITRUS JUICES

V. J. Senn, N. B. Rushing, and T. J. Kew

Southern Utilization Research Branch
Citrus Products Station, Winter Haven, Florida

The production of diacetyl and/or acetyl methyl carbinol in citrus products can be ascribed to the activities of certain species of lactic acid bacteria, particularly Lactobacillus brevis, Lact. plantarum, Leuconostoc dextranicum, and Leuco. mesenteroides, strains of which have been isolated from spoiled orange concentrate. The growth rates of strains of these bacteria, as affected by pH and by concentration of orange juice solids, have been reported previously (1). The present discussion is a preliminary report on a study designed to determine the factors affecting both the synthesis and destruction of diacetyl by these organisms.

In 20° Brix orange concentrate at an initial pH of 3.8 it was found that the ability to produce diacetyl differed widely between these species. Diacetyl as high as 400 ppm. was produced by Lact. brevis. The peak of diacetyl content tends to coincide with the midpoint of the logarithmic phase of the organism's growth curve. Continued growth results in nearly complete destruction of diacetyl.

Experiments with semi-synthetic medium have shown that diacetyl is produced from citric acid and pyruvic acid, but not from glucose, fructose, sucrose, lactic acid, malic acid, succinic acid, or fumaric acid. Attempts to identify intermediates are in progress.

While diacetyl is an undesirable constituent of citrus juices, bitterness, if not excessive, is a desirable flavor in grapefruit products. It is well recognized that naringin is the principal bitter tasting component of grapefruit juice; however, the fact that bitterness decreases through the season while naringin increases suggested a series of experiments to determine the nature of the minor constituents with particular reference to those contributing to flavor. The emphasis to date has been on the water-soluble, nonvolatile components.

Two-dimensional paper chromatography indicates that there is a minimum of 15 compounds detectable, other than sugars and acids. Three of these react to alkali as do phenolic flavonoids. The others appear as fluorescent spots under long wave ultra-violet light.

Extraction of grapefruit serum powder with a series of solvents of increasing polarity appears to offer the greatest promise of separating sugars from the minor constituents. Some success has been achieved at separation of these several components on a micro scale with solvent partition, column chromatography, and countercurrent distribution. A few crystalline mixtures have been obtained, but to date only naringin has been identified.

Publication: (1) Growth Rates of Lactobacillus and Leuconostoc Species in Orange Juice as Affected by pH and Juice Concentration, N. B. Rushing, M.K. Veldhuis, and V. J. Senn, Applied Microbiology, 4, 97-100 (1956).

COMPOSITION OF COMMERCIAL, SEGMENT, AND PEEL JUICES OF FLORIDA ORANGES

Lyle J. Swift and M. K. Veldhuis

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Citrus Products Station, Winter Haven, Florida

Excessive extractor and finisher pressures are generally considered to be related to gelation and clarification problems in orange concentrates, but little was known of possible effects on flavor, titratable acidity, pH, density, and soluble solids, as well as on the contents of sugars, soluble pectic substances, flavonoids, etc. The present study was undertaken to show what effects might be expected if increasing pressures incorporated appreciable amounts of peel juice in commercial orange juice.

Samples were collected weekly throughout the season and all were taken at one plant. At each sampling, a portion of the commercial juice was taken as it came from the finisher. Also taken were a few oranges from which segment juice was later obtained by gentle pressure. Peel-juice samples were collected at the same time and were obtained as the effluents of peel-oil centrifuges. All samples were brought immediately to the laboratory and promptly frozen and stored until the collection was complete. At that time all samples were thawed, filtered, filled into 4-oz. cans, refrozen, and stored until needed for analysis.

Soluble solids and specific gravity increased in all juices throughout the season and were almost always highest in that of the peel. Acidity remained fairly constant in all juices, but was always lowest in peel juice. As a result, the Brix-acid ratio of peel juice was at all times much the highest. Reducing sugars, sucrose, and total sugars increased in all juices as the season progressed. In the early part of the season peel juice was highest in reducing sugars and lowest in sucrose, but during the latter part of the season none of the juices exceeded the others consistently in any type of sugar. Soluble pectic substances were always highest in peel juice and were particularly high at the end of the season. Ascorbic acid was always highest in peel juice, particularly early in the season, but the content decreased in all juices as the season progressed. The flavonoid contents of commercial and segment juices held fairly constant but that of peel juice was much higher and more erratic, trending downward as the season progressed. The diacetyl contents of all types of juice rose as the season advanced, but that of peel juice rose most rapidly and was at all times the highest. Viscosities, in general, varied in much the same manner as did soluble solids. Color was almost constant in all juices for the entire season, but was much higher in the peel juice. The reverse was true with fluorescence, the peel juice giving much the lowest values. In spot checks, segment juice averaged 0.45% ash and 0.072% N while peel juice averaged 0.51% ash and 0.095% N. Peel juice

could be definitely detected when added to good-quality concentrate in the amount of 3%. Recent work has shown that while flavonoids contribute to the bitterness, it does not seem likely that they account for all of it. There is evidence that a very bitter substance is present in small amount that is neutral in reaction and perhaps volatile in steam.

Publication: L. J. Swift and M. K. Veldhuis, Composition of Commercial, Segment, and Peel Juices of Florida Oranges, J. Agr. and Food Chem. (in press).

FIVE-YEAR STORAGE OF FROZEN CONCENTRATED ORANGE JUICE

T. J. Kew

Southern Utilization Research Branch
Citrus Products Station, Winter Haven, Florida

The purpose of this study was to follow the changes in commercial frozen concentrated orange juice during long storage at several constant temperatures. A preliminary report, listed below, was made after 3 years' storage.

Ten cases of the regular commercial product were secured from each of the 4 largest concentrating plants in Florida. Each lot consisted of consecutive cans collected from the canning line. Storage temperatures were 35°, 20°, 15°, 10°, 5°, and -4°F.

At appropriate intervals cans were withdrawn and examined for cloud stability, gelation, flavor, vitamin C retention, and condition of the can. When cans stored at a particular temperature were judged to be undesirable in flavor and appearance, examinations for that temperature were concluded.

The average time required in storage for the cloud density to drop to half its initial value was 2 days at 35°F. and 15 days at 20°F. At 15°F. the range was 17.5 to 83 days. A sharp break in the data occurred at 15°F., and below this temperature greater stability was observed. The range at 10°F. storage was 184 to 686 days. At 5°F. the values were 550, 615, 1450, and more than 1825 days. At -4°F. all samples retained virtually their initial cloud density values during the 5 years in storage.

The 4 brands showed definite gel lumps within 48 days at 15°F. and sooner at higher temperatures. However, a sharp change in the rate of gelation was observed at 15°F.; below this temperature gels developed slowly. At 10°F. all samples gelled between 425 and 721 days. At 5°F. gelation was observed in 3 samples at 576, 715, and 1121 days. One sample never developed gel lumps during 5 years. At -4°F. no gelation was observed.

Fully enamelled cans were in good condition after 5 years' storage at 10°, 5°, and -4°F. Cans with plain tin ends showed slight etching of the plain tin ends at -4°F. with more severe etching at the higher temperatures. Cans without interior enamel showed slight etching of 10°F. samples.

No measurable difference in ascorbic acid content was found in any one sample between portions stored at temperatures of 10°, 5°, and -4°F., and the values found in the concentrates reconstituted after 5 years were within the normal range for fresh orange juice. It was ascertained by AOAC tests that reducing ions, such as ferrous and stannous, were not present in such quantity as to invalidate the dye titration for ascorbic acid.

After 5 years' storage at 10°F. all samples were definitely off-flavor. After 5°F. storage, opinion was divided on two brands; some tasters felt they were unacceptable while others reported the flavor to be good. All agreed that one brand was good, and that the fourth was definitely off-flavor (apricot or scorched). All samples stored at -4°F. were in excellent condition; no one detected an off-flavor.

Publication: Changes in Commercial Frozen Orange Concentrate Stored at Several Temperatures, T. J. Kew, Florida State Hort. Soc., 68, 167-170 (1956).

IN-PACKAGE GENERATION OF AMMONIA FOR FUMIGATION CONTROL OF THE BLUE-GREEN MOLDS DECAY OF CITRUS FRUITS

, F. A. Gunther, M. J. Kolbezen, R. C. Blinn,
J. H. Barkley, and E. A. Staggs

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Ammonia gas is fungicidal to a number of mold organisms, including the blue-green molds Penicillium digitatum Saccardo and P. italicum Wehmer, which commonly may attack citrus fruits after harvest and during shipment and storage. Since bulk fumigation of citrus fruits with this gas may not always afford the long-term protection sometimes required under commercial conditions, in-package generators of ammonia have been developed to maintain for long periods critical concentrations of the gas ambient to the fruit packed in standard vented or non-vented cartons.

These generators, which are activated by the highly humid environment within a carton of actively respiring citrus fruits, are of two basic types. The first type involves the simple hydrolysis of a hygroscopic ammonium salt of a weak organic acid, such as diammonium succinate, whereas the latter type utilizes the moisture-initiated reaction between a dry ammonium salt of an inorganic acid and an alkaline material such as soda ash. Many formulations incorporating these and related reactants have been evaluated in terms of physicochemical behavior as related to biological performance under both laboratory and commercial conditions (all biological testing has been under the auspices of Dr. L. J. Klotz, U.C.R.).

One of these formulations is currently being produced commercially as "Winn-Mat Fungicide No. 3" (Winning-Peplow, Inc., Los Angeles) in the form of nine 32-grain pellets of an ammonia-generating mixture encased in a special porous absorbent paper mat of long shelf-life. Details of the research program resulting in this mat will be presented.

STUBBORN DISEASE IN GRAPEFRUIT

J. B. Carpenter

Horticultural Crops Research Branch,
Date Field Station, Indio, Calif.

The disorder of citrus known as stubborn disease (also acorn disease, blue nose, pink nose, crazy top) has been recognized in California for nearly 40 years. Prior to 1954, stubborn had been described in Washington navel orange, Valencia orange, and Marsh grapefruit, and a specific virus had been designated as the causal agent, based on tissue transmission studies. Now, after 2 years of further study, the exact nature and compass of stubborn are less well defined than before. The following discussion will deal with this disease in grapefruit except as noted, for its potential effect on processing is perhaps greatest in that crop.

The real economic importance of stubborn is unknown. Nevertheless, in otherwise well managed groves, stubborn is currently held responsible for dwarfed worthless trees (free of other obvious disorders) and is believed to be an important factor in declining fruit size in bearing groves after the fifteenth to twentieth year. If these assumptions are correct, stubborn not only influences the amount of grapefruit unsatisfactory for marketing as fresh fruit and which must, therefore, be processed or discarded, but perhaps also the quality of the processed fruit.

Fruit symptoms have been more useful than tree condition in the identification of stubborn, for diverse factors may cause tree symptoms similar to those associated with stubborn, including unthriftiness, dwarfness, twigginess, dieback, off-bloom, and defoliation. The principal fruit symptoms comprise one or more of the following: acorn shape, including apparent modifications of this rind condition; blue albedo; lateral compression; a pronounced bitter off-flavor in blued fruits; and, in very small mature fruit, a thick fibrous dull-colored albedo.

Acorn disease in the Washington navel orange was early associated with stubborn disease, through the recurrent appearance of the unique acorn-shaped fruits in navel trees with a stubborn aspect. Acorn-shaped fruit is still considered to be the most certain indicator of stubborn. In grapefruit, acorn shape and blue albedo are sometimes found together. Typical stubborn grapefruit trees often bear large numbers of blued fruits. Thus, blue albedo came to be regarded as a good indicator of stubborn in grapefruit. The incidence of blued fruits in Marsh grapefruit has been from 1 to 25 or more fruits per tree in up to 60 to 70% of the trees in bearing, well-kept groves.

During the past 2 years, acorn-shaped fruit and blue albedo have been used as the principal diagnostic characters of this disease. Based on these and other symptoms, stubborn has been identified in the principal citrus-growing areas of the United States and in the following numbers of

varieties: white grapefruit, 11; pink or red grapefruit, 15; tangelo, 4; and orange, 7.

An urgently needed tool in research on stubborn is a reliable and relatively quick indicator or index plant. Twenty-eight varieties and species of citrus, inoculated with a severe strain of stubborn, showed neither foliage nor stem symptoms during 18 months of observation. These will remain under observation, while further exploratory studies on indicators are made. Concurrently, collections of stubborn are being indexed for tristeza, psorosis, xyloporosis, cachexia, vein enation, exocortis, and Rangpur lime disease to see if (a) they commonly carry one or more of these diseases, or (b) some collections are free of all these viruses.

Furthermore, the chemical nature of the blue stain in the albedo and the comparative chemical composition of normal and blued fruits must be determined to assist investigations on the physiological effects of stubborn disease.

Recently, H. Z. Hield of the California Citrus Experiment Station found an unusual amount of the blue albedo in old-line Marsh grapefruit trees that received growth regulators as sizing sprays. Also, blue albedo has been found in the Frost Nucellar Marsh grapefruit and in large seedling trees of Jochimsen grapefruit. These observations raise many questions as to (a) the cause of blue albedo and (b) its specificity as an indicator of stubborn disease. Blue albedo may be only a characteristic but nonspecific symptom, further complicating the study of this disorder.

STUDIES ON DETERMINING SOLUBLE SOLIDS IN CITRUS JUICES BY THE REFRACTOMETER

W. C. Scott and D. A. Morgan

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Citrus Products Station, Winter Haven, Florida

Refractometer scales now being used in the citrus industry are based on the refractive indices of pure sucrose solutions. The only nonsucrose constituent of citrus juices for which a correction is applied is citric acid. Stevens and Baier, in 1939, determined the refractive index and Brix of solutions of known citric acid content, and prepared tables of correction factors for refractometer readings. They recognized, but did not develop data on, the effect upon refractive index of invert sugar, ash, insoluble solids, and essential oils.

In this study efforts are being made to determine the magnitude of the effect of these and other constituents on refractive index, and to establish correction factors which will permit a more accurate estimation of true soluble solids with the refractometer. Known constituents of citrus juices were dissolved in water and/or sucrose solutions and their refractive indices measured. Those whose refractive index was found to vary appreciably from sucrose solutions of equivalent concentration were citric acid, the amino acids, fructose, and potassium citrate. Citric acid and fructose were found to indicate lower concentrations on the refractometer sugar scale than their known concentrations; therefore positive correction factors are required. Amino acids and potassium citrate require negative corrections.

The effect on refractometer values of insoluble material, or pulp, was found to be greater than would be expected from its mass alone. The negative correction for pulp is greater than the positive correction for citric acid in orange or grapefruit juices and concentrates.

As a basis for juice products of known composition, citrus powders were analyzed for moisture content by the Karl Fischer method, for water-insoluble solids, for acid by titration, for nitrogen by the Kjeldahl method, and for ash by ignition. Soluble solids and total solids were estimated by difference. These powders were reconstituted to juices of various concentrations and their refractometer values determined. Upon application of the corrections tentatively established, refractometer values were converted to within 0.4% of true soluble solids.

Because of difficulties in preparing juice samples of known composition from powders, another approach became desirable. A rapid method employing alcohol and acetone was developed for estimating insoluble solids, as well as a vacuum technique for determining total solids. Experimental

orange juice concentrates were examined by these methods, and corrections for pulp and for soluble constituents in proportions reported to occur in orange juice were applied to their refractometer values. Corrected refractometer values averaged within 0.5% soluble solids by the vacuum oven, which is significantly closer than the 1.9% average difference when acid corrections alone had been applied.

Much of the work on development of correction factors is being verified by repetition and extension. Also, their validity is being tested by comparison of refractometric evaluation and extensive analyses of more than 30 commercial and experimental concentrates.

EFFECTS OF FINISHER PRESSURE ON CHARACTERISTICS OF VALENCIA ORANGE CONCENTRATE

O. W. Bissett and M. K. Veldhuis

Southern Utilization Research Branch
Citrus Products Station, Winter Haven, Florida

The purpose of this investigation was to study the effect of varying the finisher pressure and heat treatment temperature in the preparation of Valencia orange juice for concentration.

Four lots of Valencia oranges were extracted with different finisher pressures. The percentage of finisher wastes decreased and juice yields increased with increased finisher pressure. No differences were observed in extracted juices in Brix, acid, cloud, peel oil, total pectin, or flavonoid values, but there were small increases in suspended solids, pectinesterase, and soluble pectin with increased finisher pressure.

Heat-treated and unheated juices were concentrated to 55° Brix and cut back to 42° Brix with unheated juice. The pectinesterase data on these products followed the expected trend of reduced activity with increased treatment temperature but did not indicate a change with increased finisher pressure. There was a slight increase in soluble pectin with increased finisher pressure, and the heat-treated concentrates contained more soluble pectin than the unheated controls. There was no appreciable change in flavonoid values with increased finisher pressure or heat treatment. Increases in viscosities of the concentrates with increased finisher pressure were pronounced while heat treatments did not affect viscosity values. Visual evaluation for cloud stability in 40°F. storage showed that only 3 samples retained their cloud for as much as one week. Of these, the product of 6-pound finisher setting and 170°F. heat treatment was unstable after a 2-week storage and the product of 5- and 6-pound settings and 190°F. heat treatment remained stable for 5 weeks.

Loss of cloud as indicated by increased light transmission was measured on products stored at 40°F. In all controls loss of cloud was very rapid. Heat treatment at 150°F. provided cloud stability for only a few days and treatment at 170°F. provided only slight additional stability. Concentrates prepared from juices processed at 5- and 6-pound finisher settings and heated to 190°F. were stabilized for 5 weeks or longer while products of similar heat treatments processed at 7- and 8-pound finisher settings were only slightly more stable than those heated at 170°F.

No marked differences in flavor were noted by reason of the different finisher settings used.

From a practical syandpoint, the principal effect of increased finisher pressure was a decrease in cloud stability and at the higher pressures cloud was not completely stabilized even with 190°F. heat treatment. The increased viscosities of the concentrates might cause difficulties in handling the product in the evaporator.

Publication: Effects of Finisher Pressure on Characteristics of Valencia Orange Concentrate, O. W. Bissett and M. K. Veldhuis, Proceedings Florida State Hort. Soc., 69, 1956 (in press).

IDENTIFICATION OF THE CONSTITUENTS OF LEMON OIL

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Western Utilization Research Branch, Fruit and
Vegetable Chemistry Laboratory, Pasadena, Calif.

Although the chemical composition of lemon oil has been examined by many workers in this country and in Europe, much of the information appears to be inconclusive because it was based on relatively drastic methods of separation, or in some instances merely by inference. Generally, separations were made by vacuum distillations and chemical-group reactions, both of which are known to cause degradation, polymerization, or rearrangement of highly reactive components.

Investigations of the chemistry of lemon oil have been undertaken at the Pasadena Laboratory by applying the less drastic and more efficient chromatographic techniques to the initial fractionations. Information from these studies is being used in the development of methods for analyzing oils, establishing the identity of authentic oils, and studying the processing, seasonal and geographic variations and differences in maturity affecting the composition of lemon oils.

Lemon oil has been initially separated on silicic acid columns into 20 composited fractions homogeneous to silicic acid adsorption, but probably in many instances containing more than one component. The volatile components in these fractions can be further separated by vacuum distillation or preferably by gas-liquid partition chromatography. Work on the volatiles in lemon oil is in the preliminary phases.

When composited fractions obtained from silicic acid columns were reduced in volume and stored at refrigerator temperatures, many of them deposited crystalline solids. These have yielded to date 8 individual crystalline compounds, 3 of which have been identified as ethers of coumarins and 5 as ethers of the furocoumarin, psoralen. The 3 ethers of coumarin impart a blue fluorescence to lemon oil when exposed to ultraviolet light, and all 8 compounds in composite account for the characteristic spectral absorption peak of lemon oil at around 315 millimicrons. A recent AOAC method for identifying authentic lemon oils is based on this absorption peak.

The 3 blue-fluorescing compounds have been identified as 5,7-dimethoxycoumarin (limettin),5-geranoxy-7-methoxycoumarin, and a 5,7-diether of coumarin only partially identified because of its presence in only trace amounts in the oil. Prior to this work 5,7-dimethoxycoumarin was the only coumarin compound identified in lemon oil; no furocoumarins had been previously reported.

Of the 5 non-fluorescing furocoumarins the 2 in relatively high concentration have been identified as 5-geranoxypsoralen and 8-geranoxypsoralen, the third as 5-methoxy-8-(3-methyl-2,3-dihydroxybutoxy) psoralen, while 2 of the compounds appear to be structurally related to 5-geranoxypsoralen

A semi-micro analytical method has been devised for measuring concentrations of the individual coumarins in lemon and other citrus oils.

The coumarins found in whole lemon oil were absent in distilled lemon oil and the oils of bitter orange, sweet orange, and grapefruit. Although present in lime oil, they were found in different proportions.

In order to obtain coumarin compounds for comparison with those in lemon oil, other citrus oils were similarly analyzed. Oil of bergamot, previously studied by Spath and co-workers, was used as a source to obtain 5-methoxy and 5-geranoxypsoralen; and lime oil, examined by Caldwell and Jones, to obtain 5-geranoxy-7-methoxycoumarin, 5,7-dimethoxycoumarin, and 5,8-dimethoxypsoralen. In isolating these compounds from oil of bergamot and lime oil we also identified, as new compounds, 5-geranoxy-7-methoxycoumarin in oil of bergamot and 5- and 8-geranoxypsoralen in lime oil.

An examination of grapefruit oil and bitter orange oil yielded only one compound useful for comparison, i.e., 5-methoxypsoralen (bergapten). However, several new coumarins and flavones were found. In grapefruit oil were found 7-geranoxycoumarin, an unidentified 5-position psoralen ether related to 5-geranoxypsoralen, and the flavone, tangeretin. Previously, 7-hydroxycoumarin had been reported in grapefruit oil (probably an artifact resulting from hydrolytic cleavage of the 7-geranoxycoumarin during vacuum distillation). In bitter orange oil were found 7-methoxy-8-gamma, gamma-dimethylallylcoumarin (osthol), 5-methoxypsoralen, tangeretin, nobiletin and an unidentified flavone. Auraptene, previously reported to be in orange oil, was not found.

SEPARATION AND IDENTIFICATION OF SOME TERPENES BY GAS PARTITION CHROMATOGRAPHY

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In past years, distillation was the method normally employed for the separation and analysis of the volatile constituents of natural products. The recent development of gas partition chromatography has proved to be of inestimable value for the separation and identification of essential oil constituents.

An all-glass apparatus will be described which employs a commercial polyethylene glycol as the liquid phase and a crushed Celite firebrick as its stationary support. The carrier gas employed in this study was helium, since it is quite inert and provides excellent response characteristics in the katharometers employed. The separations were carried out at column temperatures up to 180°C., permitting the use of atmospheric pressure at the outlet. The apparatus can be readily adapted to a simple fraction collector. Thus the physical and chemical examination of the individual fractions separated by the column can be readily achieved.

The time required for a given component to reach the exit of a gas partition chromatographic column is a particular characteristic of that component for a given set of experimental parameters. This so-called retention time is a function of a number of variables. In order to decrease the number of variables a new term was introduced, retention volume. The retention volume is the product of the volume of gas emerging from the column outlet in unit time and the time that elapses before the center of a zone emerges from the column. These values can be calculated with considerable accuracy and are used for the identification of components in much the same manner as other physical constants are employed.

Results obtained with this apparatus for some authentic samples of terpenes reported to occur in commercial cold-pressed lemon oil are presented in the form of retention volumes and plots of vapor concentration versus time.

A simple method will be described for the estimation of the theoretical plate efficiency of a gas partition chromatographic column.

1/ Employed by Lemon Products Advisory Board under a Memorandum of Understanding.

NITROGENOUS CONSTITUENTS IN CITRUS JUICES--EFFECTS OF PROCESSING AND STORAGE

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Investigations of the nitrogenous constituents in citrus juices have been concluded with a study of the changes in 7 free amino acids and other nitrogenous fractions in Valencia orange juice during processing and storage. Processing was performed under a range of conditions comparable to those employed commercially, after which the samples were stored for 5 months at temperatures of 0°, 40°, 70°, and 100°F. The juice products were evaluated in terms of flavor, browning, cloud stability, and their content of aspartic acid, glutamic acid, alanine, serine, arginine, proline, and gamma-aminobutyric acid. In addition, representative samples were analyzed for ammonia, amide, amino, and total nitrogen.

Cloud stability was maintained in those juices which had been heated for 4 minutes at 190°F., 2 minutes at 200°F., and longer times at these or higher temperatures. At the lower time-temperature relationships, cloud stabilities decreased slightly during storage for 5 months at temperatures between 40° and 100°F. Browning of the juices (measured in filtered juices) did not change during processing or during the ensuing storage for 5 months at 70°F. and lower temperatures. Browning increased markedly within 3 months during storage at 100°F. Flavor retention was very good in samples stored at 0°F. During storage at 70°F., flavor quality decreased gradually but retained an acceptable quality even after 5 months. The development of serious off-flavors was very rapid during storage at 100°F., becoming barely acceptable within one month.

In contrast to these marked changes in browning and flavor during storage at elevated temperatures, no significant changes were observed in the levels of the 7 amino acids. Therefore, it may be concluded that the free amino acids, with which this study was concerned, are not directly related to the darkening and development of off-flavors in pasteurized single-strength Valencia orange juice. However, marked changes were observed in the amino, ammonia, and total nitrogen of the juices during both processing and storage. This suggests that nitrogenous constituents other than those studied in the present investigation may be associated with the deterioration of orange juice. The 7 amino acids assayed account for 86% of the total nitrogen and 71% of the amino nitrogen and compose approximately 4% of the total solids in the filtered orange juice. Therefore, 14% of the total nitrogen and 29% of the amino nitrogen remain to be accounted for in terms of other nitrogenous constituents including proteins, betaines, trace amino acids, asparagine, glutamine, cysteine,

and glutathione. The latter 4 compounds are among the most labile of the nitrogenous compounds present in orange juice. It would be of interest to determine their roles in the deterioration of orange juice and to determine whether they are associated with the variations observed in amino, ammonia, and total nitrogen during processing and storage. However, satisfactory methods are not available for the estimation of these compounds in citrus juices.

Publication: Rapid Procedure for Estimation of Amino Acids by Direct Photometry on Filter Paper Chromatograms, L. B. Rockland and J. C. Underwood, Analyt. Chem., 28, 1679 (1956).